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LYMPHOID NEOPLASIA

Comment on Morin et al, page 1256

The sequence of events in diffuse large B-cell lymphoma

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In this issue of *Blood*, Morin et al report their findings on complete genome sequence analysis of primary diffuse large B-cell lymphoma (DLBCL) primary tumors and cell lines and reveal novel somatic point mutations, rearrangements, and fusions.[1] Importantly, they also demonstrate the temporal acquisition of mutations, providing insight into the evolution of mutations occurring in DLBCL.

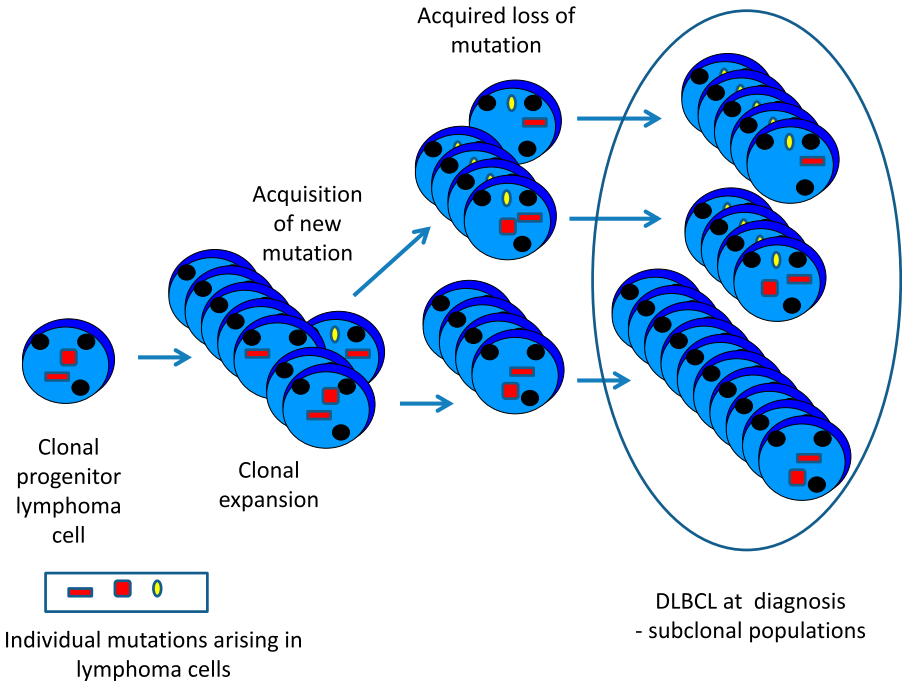
DLBCL is a heterogeneous disease with subtypes defined by distinct molecular signatures and clinical outcomes. Recent studies using massively parallel sequencing have demonstrated that DLBCL molecular subtypes also harbor distinct repertoires of somatic copy number alterations and single nucleotide variants (SNVs).[2,3] These studies also highlighted alterations in distinct molecular pathways implicated in disease pathogenesis, including the role of histone modifications in the germinal center B-cell (GCB) subtype[4,5] and B-cell receptor and NF-κB signaling in the activated B-cell (ABC) type.[6] Identification of such pathways opens new avenues for attack to treat more aggressive lymphomas[7] and provides new insight into lymphoma pathogeneisis.[8] It is noteworthy that in the present study, there was little correlation between tumor subtype and the number of genomic rearrangements, as the 5 most highly rearranged cases includes 3 ABC cases and 2 GCB cases. However, the type of mutations that occur differs between GCB and ABC subtypes, and in their manuscript the authors demonstrate somatic mutations affecting each of 3 separate genes (*GNAI2*, *S1PR2*, and *GNA13*) that cooperate in ρ-mediated B-cell homing and indicate that these genes are enriched or occur solely in GCB DLBCL.

This manuscript uses whole-genome sequencing (WGS) rather than whole-exome sequencing or RNA-seq. WGS provides the opportunity to determine the pattern, frequency, and location of somatic mutations across the entire tumor genome. It also has the potential to detect regulatory mutations that are not detectable using whole-exome sequencing approaches. The authors show here that mutations do not occur in nonrandom locations but are enriched near transcription start sites. Nonexonic mutations near the transcription start sites of genes are obviously of interest, but in the B-cell malignancies, recurrent promoter mutations are difficult to differentiate from the process of acquired somatic hypermutation (aSHM), as previously reported by this group.[9] However, the present study also reveals mutations in new genes of interest and previously undescribed mutations in individual cases, as well as recurrent mutations. The average mutation load determined by WGS in DLBCL is well above the level previously determined using whole-exome sequencing. After correcting for contamination with nontumoral cells, the authors report an average of 12 086 somatic mutations, amounting to a frequency genome-wide of 4.21 mutations/megabase and 205.6 (range, 35-400) nonsilent mutations per genome. The pattern of rearrangements and copy number alterations in some of the highly rearranged genomes is consistent with a process of chromothripsis.

WGS also has the advantage of being more sensitive to splice-site mutations. Eleven of the newly detected genes had mutation signatures indicative of tumor suppressor function with mutations at splice sites or producing a truncated protein. Mutation hot spots are indicative of potential oncogenes, and these were observed in a subset of the genes detected.

Several of the noted genes have signatures for aSHM, and these hot spots more likely reflect the preference of activation-induced cytidine deaminase for certain sequence motifs rather than evidence of selection. However, the remaining recurrent hotspots inconsistent with aSHM include those with known dominant-acting mutations in lymphoma, including *MYD88*, *CARD11*, *CD79B*, and *EZH2*, as well as *TBL1XR1*, *MEF2B*, *FAT4*, *PKD1*, *NLRP5*, and *DSEL*, the function of which has not been elucidated.

The cohort of patients studied is relatively small. High-throughput studies usually provide either high coverage and low sample number or lower coverage and more samples. The present study provides 30 times coverage of more than 40 primary, previously untreated DLBCLs and 13 cell lines. There will always be criticism of looking for higher coverage and higher sample numbers, but the number of somatic



Schematic representation of use of mutation analysis to determine cell of origin in lymphoma. With cancer evolution, there is acquisition and loss of mutations in subclones of the lymphoma. The use of WGS and calculation of allele frequency allows determination of putative lineage.

mutations identified supports this level of coverage. Results from such studies will be available once costs decrease. The heterogeneity of DLBCL makes it likely that such studies will unearth additional novel mutations.

WGS also has the advantage of allowing confirmation of the breakpoints that underlie fusion transcripts. The authors searched for evidence of the breakpoints underlying each of the fusions in both the tumor and matched normal genomes. Notably, 5 of the fusions had breakpoints detected in both the tumor and matched normal genomes and were therefore inherited events. Another 9 events had no breakpoint detectable in either the tumor or matched normal genomes. These may either represent artifacts of the technology or simply represent cases in which the breakpoint was insufficiently covered in the genomes.

Emphasis is placed here on the discoveries made possible by WGS, including not only the novel targets of point mutation but also the timing of copy number amplifications. They determined allele fraction from deep resequencing as well as loss of heterozygosity and copy number variation information to determine the cellularity of each somatic SNV. This allows direct comparison between SNVs in a single tumor and infers the order in which these were acquired, with subclonal variants representing mutations acquired later in tumor evolution. The putative temporal ordering of mutations then can be assessed in the evolution of individual tumors as shown schematically in Figure 1. They demonstrate that a large number of somatic mutations are subclonal, including many of the well-known driver mutations such as, surprisingly, hot spot mutations in *EZH2*, *MYD88*, *CARD11*, and *CD79B*. The studies on temporal ordering provide evidence that acquisition of driver mutations continues to occur during tumor progression, in addition to very early in tumorigenesis.

Overall, therefore, the results reported here reveal novel mutations, suggest evidence for chromothripsis in DLBCL, identify new fusion transcripts, and make an attempt at establishing the chronological order of the many genetic events involved in DLBCL. Why should the nonexpert reader of *Blood* care about this? It is by these types of studies[1,10] that mutations occurring in the potential lymphoma stem cells can be identified. Targeting mutations arising in the putative cells of origin will be of increasing importance. Once we are able to identify molecular pathways involved in these early events of tumorigenesis, targeted therapies aimed at killing lymphoma stem cells will be the way to prevent relapse and increase the cure rate in this disease.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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